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Toxicity of Mercuric Chloride to the Developing Rat Kidney. 1 Postnatal Ontogeny of Renal Sensitivity

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16. ABSTRACT

Although the sensitivity of the adult rat kidney to mercuric chloride has widely been reported, the degree to which this toxicant affects the developing kidney is unknown. Therefore, this study examined the effects of $HgCl_2$ on renal function during postnatal maturation. Sprague-Dawley rats were treated with a single sc injection of 5 mg/kg $HgCl_2$ on Day 1, 8, 15, 22, or 29 after birth. The effects on renal function, histology, and morphology were assessed 24, 48, and 120 hr after each treatment. Measurements of renal function included urine volume, osmolality, the ability to concentrate urine during water deprivation, urinary pH, chloride and protein content, tests for glucosuria, hematuria, and various serum chemistry parameters. Rats were killed and their kidneys processed and examined by light microscopy. The renal sensitivity to $HgCl_2$ increased throughout maturation for every parameter measured. No pups treated with $HgCl_2$ on Day 1 died, but mortality increased to almost 20% in rats treated 22 and 29 days after birth. Body weight was unaffected in Day 1 animals, but was decreased at 120 hr post-treatment in three of the other four age groups. Kidney weights were unaffected in 1- and 8-day olds, but were increased by 10 to 55% in rats that were 15, 22, and 29 days old. Urine volume was increased 48 to 72 hr following treatment at all ages. The ability to concentrate urine in response to water deprivation was compromised in all animals with the exception of those treated on Day 1 and was decreased to the greatest extent in 29-day-olds.

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Toxicity of Mercuric Chloride to the Developing Rat Kidney
I. Postnatal Ontogeny of Renal Sensitivity

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Toxicity of Mercuric Chloride to the Developing Rat Kidney. I. Postnatal Ontogeny of Renal Sensitivity. DASTON, G. P., KAVLOCK, R. J., ROGERS, E. H., AND CARVER, B. (1983). *Toxicol. Appl. Pharmacol.* **71**, 24–41. Although the sensitivity of the adult rat kidney to mercuric chloride has been widely reported, the degree to which this toxicant affects the developing kidney is unknown. Therefore, this study examined the effects of $HgCl_2$ on renal function during postnatal maturation. Sprague-Dawley rats were treated with a single sc injection of 5 mg/kg $HgCl_2$ on Day 1, 8, 15, 22, or 29 after birth. The effects on renal function, histology, and morphology were assessed 24, 48, and 120 hr after each treatment. Measurements of renal function included urine volume, osmolality, the ability to concentrate urine during water deprivation, urinary pH, chloride and protein content, tests for glucosuria, hematuria, and various serum chemistry parameters. Rats were killed and their kidneys processed and examined by light microscopy. The renal sensitivity to $HgCl_2$ increased throughout maturation for every parameter measured. No pups treated with $HgCl_2$ on Day 1 died, but mortality increased to almost 20% in rats treated 22 and 29 days after birth. Body weight was unaffected in Day 1 animals, but was decreased at 120 hr post-treatment in three of the other four age groups. Kidney weights were unaffected in 1- and 8-day olds, but were increased by 10 to 55% in rats that were 15, 22, and 29 days old. Urine volume was increased 48 to 72 hr following treatment at all ages. The ability to concentrate urine in response to water deprivation was compromised in all animals with the exception of those treated on Day 1, and was decreased to the greatest extent in 29-day-olds. Urinary chloride concentration was decreased in Day-22 animals at 24 and 48 hr, and in Day-29 rats at all times observed after injection. Urinary pH was more acidic in treated suckling pups, and more basic in treated pups after weaning on Day 22. Urinary protein content was increased after exposure in all but the pups treated on Day 1. Serum creatinine was increased at 120 hr after injection in Day-8 rats, and 24 and 48 hr after injection in older rats. Glucosuria and hematuria occurred with increasing frequency as the pups matured. Histological evaluation revealed some cortical tubular dilatation in rats treated on Day 1 or Day 8; there was tubular necrosis in older rats. For all parameters observed, the neonatal kidney was largely insensitive to $HgCl_2$ toxicity; however, a trend toward increased sensitivity with increasing age was demonstrated.

Mercuric chloride is a potent nephrotoxin. In mature animals the principal site affected is the proximal tubule, where it may induce necrosis of the tubular epithelium and result in acute renal failure (Ganote *et al.*, 1974; McDowell *et al.*, 1976).

The extent of nephrotoxicity produced by $HgCl_2$ in the developing postnatal mammal is not as clear. Bidani *et al.* (1979) reported that 2-week-old rats were more sensitive to $HgCl_2$ than older rats. Wold *et al.* (1977) found that rabbits, from 5 days of age to adults, had

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similar toxic responses following $HgCl_2$ exposure regardless of age. In contrast to these reports, Kavlock (1982) found no evidence of renal toxicity in 3-, 6-, or 10-day-old rats exposed to $HgCl_2$ on postnatal Days 1 and 2.

The distribution and excretion of mercury in the neonatal rat is considerably different from that of the adult. Mercury is very slowly eliminated from the body in the first 2 weeks of postnatal life, after which the rate of excretion increases. In conjunction with this onset of excretion, the renal levels of mercury, as a fraction of the whole body burden, increase (Thomas and Smith, 1979). Whole body burdens produced by a given dose of mercury are higher in 2-week-old rats than in adults (Jugo, 1975). These findings indicate that the mercury excretion pattern in the immature animal differs from that of the adult, and that there may be an age-related sensitivity with respect to nephrotoxicity.

The present study was undertaken to investigate the effects of $HgCl_2$ on the postnatally maturing rat kidney, from birth through post weaning.

METHODS

Animals

Time pregnant Sprague–Dawley CD rats (Charles River Breeding Laboratories, Wilmington, Mass.) were received in our laboratory on day 15 of gestation (sperm positive day = day 1). The animals were individually housed on wood shavings in a bio-clean room maintained at a constant temperature (20 to 24°C), relative humidity (40 to 50%), and photoperiod (12-hr light/day). Animals were allowed access to commercial lab chow (Purina Laboratory Chows, St. Louis, Mo.) and tap water *ad libitum*.

Animals gave birth on gestation day 22. The following day was designated postnatal Day 1. To minimize genetic effects, all pups were removed on Day 1 from their dams and separated by sex. Each dam was then issued five male and five female pups selected randomly from the total pool.

Pups were injected sc on Day 1, 8, 15, 22, or 29 with 5 mg/kg $HgCl_2$ dissolved in 0.85% NaCl (3.3 μl solution/g body wt). Controls were given normal saline injections of equivalent volume. Both control and $HgCl_2$-injected groups consisted of 40 to 50 pups (from four or five litters) in each of the five age groups studied. A total of 440 pups were used in this study.

Renal Function Assessments

Renal function was evaluated 24, 48, and 120 hr post-injection (pi). Pups were isolated from the dam, their bladders voided, and urine osmolality determined using a vapor pressure osmometer (Wescor Model 5100B, Logan, Utah). To ensure fluid deprivation, pups were subsequently kept in beakers filled with wood shavings in a water bath maintained at 35°C. The urine produced after a 4-hr period of water deprivation was collected quantitatively by the method of Kavlock and Gray (1982), which involves stimulation of the perineal region with a capillary tube to initiate reflex micturition. (Quantitative collection by this method was not possible for pups older than 20 days, as these pups may void voluntarily). Blood samples were also obtained at this time by cardiocentesis. Urine osmolality was again measured. Chloride content of the urine was determined coulometrically (Radiometer Copenhagen CMT-10 Chloride Titrator, Copenhagen, Denmark). Urine pH was measured with a micro-combination probe (Microelectrodes, Inc., Londonderry, N.H.). Urinary protein was determined colorimetrically using Coomassie blue binding (Bio-Rad Protein Assay, Bio-Rad Laboratories, Richmond, Calif.). Serum and urine creatinine contents were measured colorimetrically with a commercial assay kit (Sigma Chemical Co., St. Louis, Mo.). Lastly, clinical reagent strips were used to qualitatively determine the presence of protein, glucose, and occult blood in the urine. The test strips were read photometrically by a semi-automated analyzer (Clini-Tek, Ames Co., Elkhart, Ind.).

Quantitative Urine Collection of 29-Day-Old Animals

Pups for this experiment were weaned on postnatal Day 21, placed in metabolism cages (Sybron Nalge, Rochester, N.Y.) on Day 28, and injected with 5 mg/kg $HgCl_2$ or normal saline on Day 29. The diet consisted of powdered lab chow (Purina Lab Chow) mixed with corn oil (5:1, w:v). The resultant mixture was semisolid and thus prevented contamination of the collected urine by spilled powdered diet. Water consumption, urine output, and body weight were determined daily. To measure urine concentrating abilities, these animals were fluid deprived for 24 hr beginning 96 hr pi, after which they were killed. Although this period was past the time of greatest renal damage, it was believed that the animals should not be subjected to undue physiologic stress until the termination of the experiment. Urinary parameters, osmolality, pH, chloride, protein, glucose, and blood were determined on daily urine samples.

Histology

Following the water deprivation experiment, animals were killed with an ip injection of sodium pentobarbital. Kidneys were removed, weighed, and fixed in a 5% formaldehyde, 5% acetic acid, 70% ethanol solution. The kidneys were paraffin embedded, sectioned, stained with hematoxylin and eosin, and examined by light microscopy (Experimental Pathology Laboratories, Raleigh, N.C.).

Serum Chemistry Analysis

Blood was obtained by cardiocentesis 24, 48, and 120 hr pi from pups treated on Day 22 or 29. Serum concentrations of creatinine, urea nitrogen, uric acid, glucose, total protein, albumin, total bilirubin, alkaline phosphatase, glutamic oxalacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT) were determined using an automated analyzer (Centrifichem System 400, Union Carbide Corp., Rye, N.Y.).

Statistics

Data were analyzed statistically by the general linear model procedures available on the Statistical Analysis System (SAS Users Guide, 1979). Differences between control and treated groups were compared by analysis of variance at each age and observation time. Treatment effects on kidney weight were analyzed using body weight as a covariate, thus eliminating differences in total body mass as an explanation for kidney weight differences. All data were reported as mean ± standard error of the mean.

RESULTS

Day-1 Exposure

Mercuric chloride had the least effect on renal function when given to 1-day-old pups. The treatment was not lethal to any of these pups. Neither body weight nor kidney weight was affected at any of the observation times (Table 1).

Neonates had a limited ability to concentrate urine, as 2-day-olds could only raise their urine osmolality by a mean of 148 mOsM, to 387 mOsM, during a 4-hr period of water deprivation (Table 2). Concentrating ability increased steadily thereafter. Injection with $HgCl_2$ produced polyuria 48 hr pi, as urine volume was increased by 23% relative to body weight in treated animals. Neither basal nor hydropenic urinary osmolality were affected by the treatment (Table 2).

Urinary pH was significantly more acidic 24 (5.38 vs 5.78) and 48 (5.63 vs 5.93) hr after injection on Day 1. Urinary protein, which was relatively high in control neonates, was unaffected by $HgCl_2$ exposure. Serum creatinine levels, elevated in controls at this age, were unaffected by $HgCl_2$ (Table 3).

Some minor renal lesions were found in rats treated on Day 1. Approximately half of the treated rats exhibited mild cortical tubular degeneration and dilatation by 48 to 120 hr pi. (Fig. 1).

Day-8 Exposure

As with injection on Day 1, none of the animals treated on Day 8 died as a result of the exposure to $HgCl_2$. In all other parameters, however, these animals were more affected than neonates. Although body weight was not different from controls 24 or 48 hr pi, it was reduced by more than 25% ($p < 0.001$) 120 hr pi. Kidney weight was unaffected by the treatment (Table 1).

Mercuric chloride treatment produced polyuria in 8-day-olds 24 hr pi, which became much more pronounced 48 hr pi. The increased urine volume was statistically significant (135 and 243% of control values, respectively) at both observation times (Table 2). Basal urine osmolality (at the time of separation from the dam) was unaffected by the treatment on Day 8. Urine osmolality after 4 hr of water deprivation was significantly lower in rats treated with $HgCl_2$ (72% of control value) 48 hr pi (Table 2).

Urinary pH was more acidic in $HgCl_2$-treated 8-day-olds than in controls, as was the case for neonates. In the 8-day-old rats, the pH effect was more prominent; hydrogen ion concentration increased by approximately an order of magnitude ($p < 0.001$) at all times observed (Table 3). Urinary protein levels in Day-8 control pups declined to an extremely low level. However, pups treated with $HgCl_2$

TABLE 1

EFFECTS OF INJECTION OF 5 mg/kg $HgCl_2$ ON BODY AND KIDNEY WEIGHT

Age at injection (days after birth)	Hours postinjection	No. of animals		Body weight (g)		Kidney weight (mg)	
		Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$
1	24	13	13	7.7 ± 0.4	7.6 ± 0.4	85 ± 6	89 ± 8
	48	13	14	8.6 ± 0.3	9.1 ± 0.6	99 ± 5	112 ± 9
	120	13	12	14.9 ± 1.0	14.4 ± 0.8	189 ± 10	193 ± 11
8	24	13	14	17.3 ± 0.4	17.0 ± 0.5	213 ± 5	227 ± 9
	48	13	13	20.9 ± 0.4	19.1 ± 0.7	267 ± 7	284 ± 12
	120	14	13	30.0 ± 0.4	22.2 ± 1.2[c]	397 ± 9	358 ± 16
15	24	16	16	29.5 ± 0.7	31.9 ± 0.9	369 ± 11	459 ± 15[a,d]
	48	16	14	32.9 ± 0.8	34.0 ± 1.2	406 ± 9	538 ± 18[c,e]
	120	17	14	43.5 ± 1.0	42.5 ± 1.6	571 ± 17	757 ± 48[c,e]
22	24	15	16	50.3 ± 1.7	51.3 ± 1.3	632 ± 21	777 ± 22[c,e]
	48	14	15	54.4 ± 1.3	52.1 ± 1.4	653 ± 18	880 ± 27[c,e]
	120	16	12	70.9 ± 1.8	54.6 ± 3.2[c]	790 ± 26	1205 ± 65[c,e]
29	24	11	12	88.3 ± 2.6	79.8 ± 2.4[c]	974 ± 33	1038 ± 29[d]
	48	10	13	94.6 ± 4.5	75.4 ± 2.3[c]	1040 ± 40	1175 ± 36[b,e]
	120	12	11	114.3 ± 3.8	90.6 ± 3.2[c]	1136 ± 31	1532 ± 129[c,e]

[a] Significantly different from controls by general linear models procedure, $p < 0.05$.
[b] Significantly different from controls by general linear models procedure, $p < 0.01$.
[c] Significantly different from controls by general linear models procedure, $p < 0.001$.
[d] Significantly different from controls using body weight as a covariate, $p < 0.05$.
[e] Significantly different from controls using body weight as a covariate, $p < 0.001$.

on Day 8 were proteinuric ($p < 0.05$) 120 hr pi (Table 3). Serum creatinine levels in control pups during the second week of life remained around 1.0 mg/100 ml. Creatinine levels in $HgCl_2$-treated pups were 30 to 70% higher than controls throughout the experimental period, but this increase was statistically significant (1.65 vs 0.97 mg/100 ml) only 120 hr pi (Table 3).

The pups were qualitatively assessed for hematuria and glucosuria. None of the control pups at any age had glucose in their urine. Blood was detected in the urine of a single 9-day-old control, but this effect was attributed to urethral injury during urine collection. Treatment related glucosuria was observed most frequently 24 hr pi at all ages. Of the pups injected on Day 8, 28.6% were glucosuric at 24 hr, 0% at 48 hr, and 7.7% (1 of 13) at 120 hr pi (Table 4).

Mercury-induced renal lesions were more pronounced in 8-day-old animals. Only 8% of the pups injected with $HgCl_2$ on Day 8 had normal renal histology. Acute cortical tubular necrosis was occasionally present 24 and 48 hr pi; however, cortical tubular dilatation was more prevalent than at Day 1 (Fig. 2). By 120 hr pi, regeneration of cortical tubular epithelium was evident.

Day-15 Exposure

The effects of exposure to $HgCl_2$ became more pronounced as the rats matured. Treatment was lethal for 5% of the pups treated on Day 15. Body weight was not reduced. Kidney weight was increased by 24% ($p < 0.05$) as compared to control values 24 hr pi, and by 33% ($p < 0.001$) 48 and 120 hr pi (Table 1).

The ability of control pups to produce a concentrated urine in response to water deprivation continued to improve. Twenty-day-old control pups produced a urine with a solute

TABLE 2

EFFECTS OF INJECTION OF 5 mg/kg $HgCl_2$ ON URINE CONCENTRATING ABILITY

Age at injection (days after birth)	Hours postinjection	No. of animals		Initial urine osmolality (mOsM)		Urine osmolality after 4-hr water depreviation (mOsM)		Relative urine volume (μl/hr/g body wt.)	
		Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$
1	24	13	13	239 ± 18	270 ± 17	387 ± 26	395 ± 26	2.9 ± 0.3	2.9 ± 0.2
	48	12	14	264 ± 20	287 ± 12	422 ± 16	410 ± 13	3.1 ± 0.3	3.8 ± 0.1[a]
	120	13	12	396 ± 41	319 ± 45	588 ± 21	561 ± 35	2.7 ± 0.2	2.8 ± 0.2
8	24	13	14	269 ± 19	244 ± 28	418 ± 21	301 ± 36	2.8 ± 0.1	3.8 ± 0.2[a]
	48	13	13	252 ± 25	261 ± 25	515 ± 31	373 ± 20	2.3 ± 0.2	5.6 ± 0.3[c]
	120	14	13	423 ± 25	509 ± 25	633 ± 18	587 ± 29	3.7 ± 0.2	4.5 ± 0.5
15	24	16	14	241 ± 31	275 ± 27	484 ± 45	477 ± 53	2.9 ± 0.3	3.0 ± 0.5
	48	16	15	423 ± 66	419 ± 50	855 ± 75	649 ± 57[a]	2.5 ± 0.3	3.4 ± 0.5[a]
	120	17	14	652 ± 103	880 ± 107[b]	1099 ± 92	1225 ± 84	1.7 ± 0.2	1.1 ± 0.2
22	24	15	15	664 ± 70	422 ± 65[b]	1045 ± 97	598 ± 94[c]	ND[d]	ND
	48	14	15	500 ± 87	344 ± 28[a]	1391 ± 108	715 ± 81[c]	ND	ND
	120	16	12	1106 ± 103	541 ± 78[c]	1689 ± 101	1142 ± 122[c]	ND	ND
29	24	11	12	777 ± 95	456 ± 31[c]	1551 ± 105	835 ± 84[c]	ND	ND
	48	10	14	1117 ± 25	365 ± 41[c]	1833 ± 167	753 ± 97[c]	ND	ND
	120	12	11	605 ± 74	806 ± 77[a]	1864 ± 116	1342 ± 155[c]	ND	ND

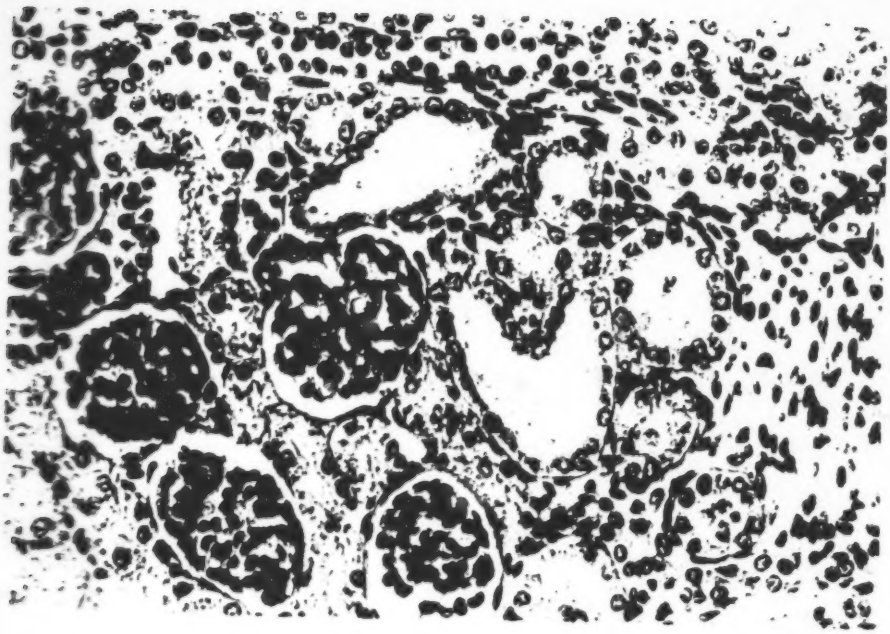
[a] Significantly different from controls by general linear models procedure, $p < 0.05$.
[b] Significantly different from controls by general linear models procedure, $p < 0.01$.
[c] Significantly different from controls by general linear models procedure, $p < 0.001$.
[d] ND, not determined.

TABLE 3

EFFECTS OF INJECTION OF 5 mg/kg $HgCl_2$ ON RENAL PARAMETERS IN PREWEANLING RATS

Age at injection (days after birth)	Hours post-injection	No. of animals		Urine pH		Urine chloride (meq/l)		Urine protein (mg/100 ml)		Serum creatinine (mg/100 ml)	
		Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$	Control	$HgCl_2$
1	24	13	13	5.78 ± 0.09	5.32 ± 0.13[a]	ND[d]	ND	4.78 ± 2.50	1.63 ± 1.02	1.08 ± 0.14	1.23 ± 0.16
	48	13	14	5.93 ± 0.18	5.63 ± 0.13[b]	ND	ND	8.06 ± 2.00	7.08 ± 1.87	0.91 ± 0.05	1.07 ± 0.07
	120	13	12	6.16 ± 0.11	5.95 ± 0.18	ND	ND	11.72 ± 2.88	10.78 ± 2.90	0.94 ± 0.07	1.08 ± 0.08
8	24	13	14	6.56 ± 0.09	5.40 ± 0.08[c]	ND	ND	6.15 ± 2.76	4.43 ± 1.14	1.50 ± 0.14	2.25 ± 0.27
	48	13	13	6.69 ± 0.17	5.49 ± 0.10[c]	ND	ND	2.08 ± 0.88	3.28 ± 0.82	0.81 ± 0.06	1.11 ± 0.06
	120	14	13	5.86 ± 0.06	4.93 ± 0.09[c]	ND	ND	0 ± 0	19.68 ± 7.88[a]	0.97 ± 0.07	1.65 ± 0.25[b]
15	24	16	16	6.35 ± 0.09	5.43 ± 0.07[c]	17 ± 2	15 ± 3	2.97 ± 0.42	5.47 ± 0.73[a]	0.67 ± 0.05	1.22 ± 0.16[a]
	48	16	14	6.03 ± 0.10	5.64 ± 0.11[a]	21 ± 5	32 ± 5	0.99 ± 0.42	2.08 ± 1.07	0.32 ± 0.03	0.85 ± 0.29[a]
	120	17	14	6.19 ± 0.17	5.96 ± 0.10	48 ± 7	68 ± 8	13.34 ± 2.19	28.94 ± 7.64[a]	0.35 ± 0.03	0.36 ± 0.04
22	24	15	15	6.62 ± 0.18	5.41 ± 0.13[c]	116 ± 14	85 ± 12	25.97 ± 3.62	45.17 ± 9.58[a]	ND[d]	ND
	48	14	15	6.02 ± 0.12	5.46 ± 0.17[b]	112 ± 12	88 ± 5[c]	12.55 ± 2.56	35.24 ± 14.56[b]	ND	ND
	120	16	12	5.81 ± 0.11	5.75 ± 0.14	209 ± 17	107 ± 22[c]	ND	ND	ND	ND

[a] Significantly different from controls by general linear models procedure, $p < 0.05$.
[b] Significantly different from controls by general linear models procedure, $p < 0.01$.
[c] Significantly different from controls by general linear models procedure, $p < 0.001$.
[d] ND, not determined.



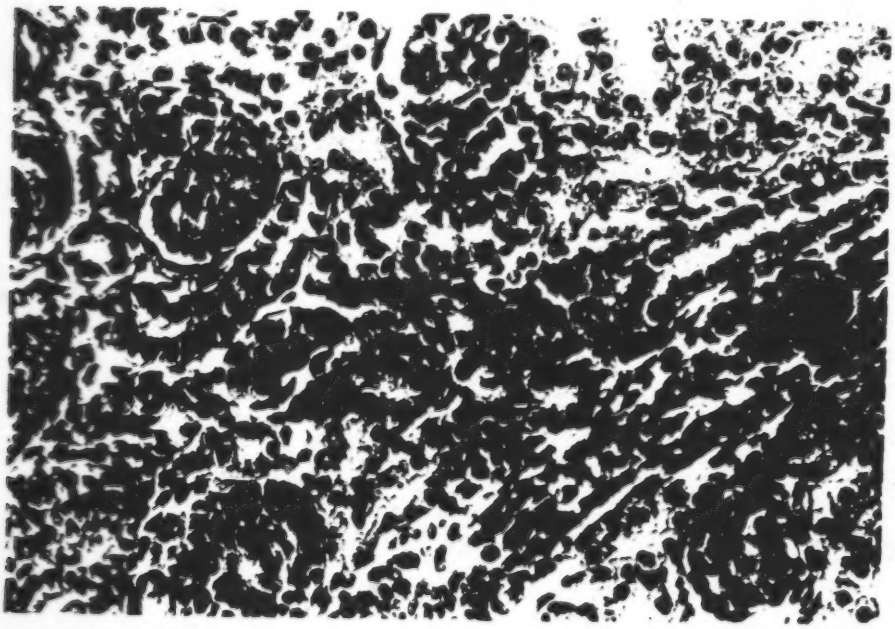


TABLE 4

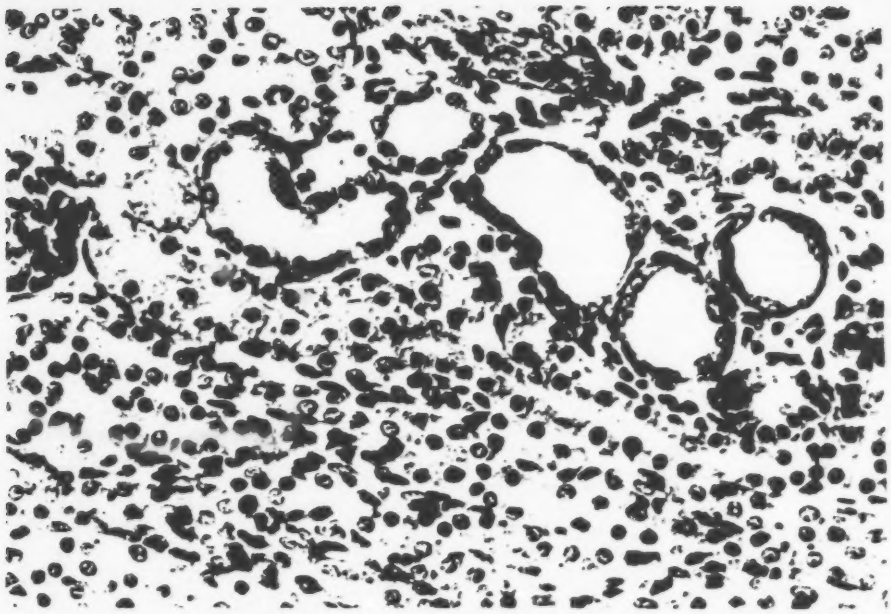
ABNORMAL URINARY CONSTITUENTS FOLLOWING 5 mg/kg $HgCl_2$ INJECTION

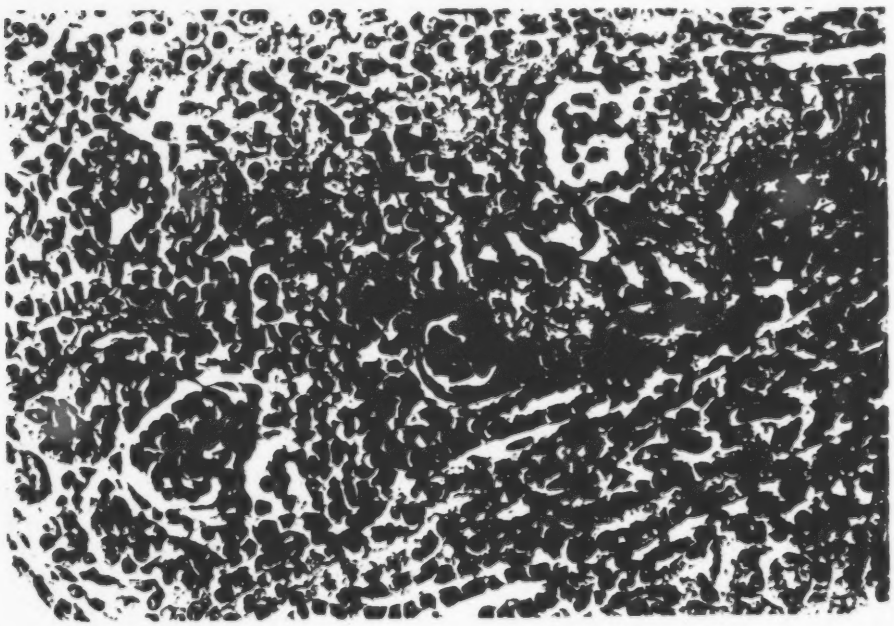
Age at injection (days after birth)	Hours postinjection	No. of animals	Hematuria (% affected individuals)	Glucosuria (% affected individuals)
Control				
8	24	13	0	0
	48	13	7.7	0
	120	14	0	0
15	24	16	0	0
	48	16	0	0
	120	17	0	0
22	24	15	0	0
	48	14	0	0
	120	16	0	0
29	24	11	0	0
	48	10	0	0
	120	12	0	0
$HgCl_2$				
8	24	14	0	28.6
	48	13	23.1	0
	120	13	7.7	7.7
15	24	16	6.3	50.0
	48	14	0	14.3
	120	14	7.1	0
22	24	15	13.3	73.3
	48	15	20.0	53.3
	120	12	0	16.7
29	24	9	77.8	88.9
	48	14	28.6	64.3
	120	11	0	0

content greater than 1000 mOsM after 4 hr of water deprivation (Table 2). Pups injected with $HgCl_2$ had a significantly increased urine volume (135% of controls) 48 hr pi. In conjunction with this increase, hydropenic urine osmolality was only 76% of the control value ($p < 0.05$) at this observation time (Table 2). Basal osmolality was unaffected at this time, but was significantly higher ($p < 0.01$) than controls 120 hr pi (Table 2).

As before, urinary pH was lowered following $HgCl_2$ exposure. This decrease was most prominent 24 hr pi, when mean urine pH was 5.43, as compared to 6.35 for controls ($p < 0.01$). The urine from treated rats was more acidic than the control urine at the other observation times, but this alteration was statistically significant ($p < 0.05$) only 48 hr pi (Table 3). Urinary chloride content, although increased 48 and 120 hr pi in treated animals,

FIG. 1. Photomicrographs of the kidney cortex from control (bottom) and $HgCl_2$-treated rats (top). The rats were killed 48 hr after injection on Day 1. In the control, histologically mature tubules and glomeruli can be seen in the right portion of the micrograph, whereas the distinctive S-shaped morphology of forming nephrons can be seen in the nephrogenic zone at the left. Note especially that the cortical tubules have a relatively small luminal diameter. In contrast, many of the proximal tubules in the $HgCl_2$-treated kidney are dilatated. Original magnification—200×; hematoxylin and eosin stain.





was not significantly different from controls (Table 3). Urinary protein content was low in controls on Days 16 and 17, but rose to a mean of 13.34 mg/100 ml on Day 20. Protein levels in treated animals of this age were approximately double the control level, a significant difference ($p < 0.05$) occurring 24 and 120 hr pi (Table 3). Serum creatinine levels in controls began to fall during this period. Day 16 rats had a mean of 0.67 mg/100 ml, and subsequently (Day 17 through Day 34, the oldest animals used) the levels were between 0.25 and 0.42 mg/100 ml (Tables 3 and 5). Creatinine levels in $HgCl_2$-exposed animals were 2 to 2.5 times higher than controls at 24 and 48 hr pi ($p < 0.05$), but returned to normal at 120 hr (Table 3).

Glucosuria was detected in 50% of the Day-15 treated animals 24 hr pi, in 14.3% 48 hr pi, and in none 120 hr pi. Two treated individuals had blood in the urine: one had 24 and one 120 hr pi (Table 4).

Rats treated with $HgCl_2$ on Day 15 had an increased incidence of histologic lesions as compared to those treated on Day 8. Only 5% of Day-15 rats had normal kidneys. Acute cortical tubular necrosis was prevalent 24 to 48 hr pi, and cortical tubular dilatation was widespread. By 120 hr pi, tubular regeneration had begun.

Day-22 Exposure

Mercuric chloride was more nephrotoxic in 22-day-old rats than in rats treated at earlier ages. Almost one-fifth of the animals given $HgCl_2$ on Day 22 died in the ensuing 5 days. Body weight was reduced significantly ($p < 0.001$) at 120 hr pi, to only 77% of control weight. Kidney weight was increased at all observation times; the increase ranged from 23% at 24 hr to 53% at 120 hr pi. This increase was statistically significant ($p < 0.001$) at all observation times (Table 1). Using covariate analysis to control for differences in body weight, kidney weight was significantly increased ($p < 0.001$) in treated animals (Table 1).

Basal urine osmolality in treated rats was significantly lower ($p < 0.05$) than control values 24, 48, and 120 hr pi (Table 2). Urine osmolality after 4 hr of water deprivation was decreased by 30 to 50% at all three times in $HgCl_2$-exposed animals ($p < 0.001$; Table 2). Urine volume was not measured in Day-22 rats as they may micturate voluntarily. Several 22-day pups were weaned and placed in metabolism cages. The data obtained from these animals was highly variable, as food and water intake in the control group was variable for several days; hence, these data are not displayed here. However, relative urine volume was consistent over time, as controls produced between 7.6 and 9.6 ml/100 g body wt/day over a 6-day period. Mercuric chloride-treated rats produced mean urine volumes of 14.9 and 20.6 ml/100 g body wt/day 24 and 48 hr pi ($p < 0.05$ and 0.01, respectively).

Urinary pH remained significantly more acidic (82 to 90% of control pH) 24 and 48 hr pi (Table 3). Urinary chloride content increased during this period, but was decreased ($p < 0.001$) from control levels 48 and 120 hr after $HgCl_2$ exposure (Table 3). Subsequent to Day 20, a small quantity of protein was a normal urinary constituent. Urinary protein levels were increased after $HgCl_2$ exposure to concentrations as much as 2 to 3 times higher than control values ($p < 0.05$) 24 and 48 hr pi (Table 3).

FIG. 2. Photomicrographs of kidney cortex from control (bottom) and $HgCl_2$-treated (top) rats. The rats were killed 48 hr after injection on Day 8. From the left to the right of the control micrograph, less to more histologically mature nephrons are present. The nephrogenic zone (not depicted here) is extremely thin at this age. The cortical tubules are normal. In the kidney from the $HgCl_2$-treated rat there is extensive dilatation of the proximal tubules. Necrosis in cortical tubules was observed at this age, but was uncommon and is not present in this section. Original magnification–200×; hematoxylin and eosin stain.

TABLE 5

EFFECTS OF INJECTION OF 5 mg/kg $HgCl_2$ ON SERUM CHEMISTRY PARAMETERS IN WEANLING RATS

Age at injection (days after birth)	Hours post-injection	No. of animals	Creatinine (mg/100 ml)	Urea nitrogen (mg/100 ml)	Uric acid (mg/100 ml)	Glucose (mg/100 ml)	Total protein (g/100 ml)	Albumin (g/100 ml)	Total bilirubin (mg/100 ml)	Alkaline phosphatase (IU/l)	SGOT (IU/l)	SGPT (IU/l)
Control												
22	24	10	0.35 ± 0.05	19.7 ± 0.7	2.4 ± 0.4	183.2 ± 10.0	4.2 ± 0.1	2.9 ± 0.1	0.50 ± 0.06	275 ± 10	182 ± 30	24 ± 2
	48	9	0.25 ± 0.06	17.5 ± 1.1	2.2 ± 0.3	191.8 ± 13.7	4.1 ± 0.1	2.9 ± 0.1	0.66 ± 0.10	338 ± 23	191 ± 31	27 ± 4
	120	9	0.31 ± 0.06	15.2 ± 0.6	1.6 ± 0.3	178.5 ± 6.6	4.3 ± 0.1	3.0 ± 0.1	0.42 ± 0.07	323 ± 26	176 ± 26	29 ± 2
29	24	13	0.42 ± 0.01	14.7 ± 0.7	ND[d]	206.6 ± 11.8	4.4 ± 0.1	3.0 ± 0.1	0.30 ± 0.05	290 ± 29	157 ± 24	24 ± 2
	48	14	0.33 ± 0.02	12.9 ± 0.6	ND	183.3 ± 5.0	4.5 ± 0.1	3.0 ± 0.1	0.38 ± 0.04	295 ± 13	182 ± 19	24 ± 1
	120	13	0.38 ± 0.03	12.5 ± 0.8	ND	186.7 ± 4.4	4.5 ± 0.1	3.0 ± 0.1	0.28 ± 0.04	235 ± 22	159 ± 14	21 ± 1
$HgCl_2$												
22	24	11	1.72 ± 0.38[c]	102.6 ± 19.3[c]	2.1 ± 0.3	166.6 ± 5.9	4.2 ± 0.1	2.5 ± 0.1[b]	0.48 ± 0.07	314 ± 28	280 ± 45	24 ± 5
	48	10	1.94 ± 0.55[c]	127.1 ± 31.6[c]	2.9 ± 0.7	233.7 ± 34.4[a]	4.5 ± 0.1	3.0 ± 0.4	0.65 ± 0.15	281 ± 35	226 ± 55	25 ± 4
	120	10	0.70 ± 0.41	41.4 ± 24.6	2.4 ± 0.3	174.8 ± 11.1	4.2 ± 0.1	2.7 ± 0.1	0.53 ± 0.08	271 ± 29	356 ± 81[c]	49 ± 11[b]
29	24	12	1.93 ± 0.28[c]	100.9 ± 15.4[c]	ND	161.4 ± 14.6[c]	4.3 ± 0.1	2.6 ± 0.1[b]	0.32 ± 0.08	327 ± 18	260 ± 31[a]	24 ± 2
	48	12	2.80 ± 0.35[c]	156.6 ± 21.0[c]	ND	178.6 ± 16.9	4.5 ± 0.1	2.6 ± 0.1[c]	0.33 ± 0.03	235 ± 22	189 ± 30	20 ± 4
	120	13	0.51 ± 0.03	31.1 ± 4.5	ND	175.4 ± 2.9	4.5 ± 0.1	2.9 ± 0.1	0.29 ± 0.03	212 ± 20	157 ± 13	22 ± 2

[a] Significantly different from control by general linear models procedure, $p < 0.05$.
[b] Significantly different from control by general linear models procedure, $p < 0.01$.
[c] Significantly different from control by general linear models procedure, $p < 0.001$.
[d] ND, not determined.

Extensive serum chemistry profiles were determined in animals injected with $HgCl_2$ on Days 22 and 29. In 22-day pups, serum cretinine and urea nitrogen were significantly higher (5 to 7 times greater than controls) at 24 and 48 hr pi. Albumin content was lower ($p < 0.01$) than controls at 24 hr pi, although total protein was unaffected. Glucose was increased by 22% ($p < 0.05$) in treated rats 48 hr pi. Serum GOT and GPT were increased ($p < 0.01$) by the treatment 120 hr pi. Uric acid, total bilirubin, and alkaline phosphatase were unchanged (Table 5).

Of the animals injected with $HgCl_2$ on Day 22, 73% were glucosuric at 24 hr, 53% at 48 hr, and 17% at 120 hr pi. Two of fifteen were hematuric 24 hr pi, and three of fifteen 48 hr pi. (Table 4).

Histologic lesions were increasingly apparent in $HgCl_2$-treated rats at this age. Acute cortical tubular necrosis was prevalent at 24 and 48 hr pi. By 120 hr pi, tubular regeneration had begun. Tubular basement membranes were lined with squamous and low cuboidal epithelium. Mineralized casts were often present in the proximal tubular lumens at this time.

Day-29 Exposure

For all parameters measured, $HgCl_2$-induced nephrotoxicity was most profound and persistent in animals treated on Day 29. The treatment resulted in slightly more than 20% mortality. Body weight was reduced by 10% 24 hr pi and by almost 25% 120 hr pi ($p < 0.001$; Table 1). Absolute kidney weight was increased by 13 to 35% ($p < 0.01$) 48 and 120 hr pi. After correcting for body weight differences by covariate analysis, it was shown that kidney weight as a function of body weight was increased ($p < 0.05$) at all observation times (Table 1).

Six control and six treated rats were individually housed in metabolism cages. The treated rats were polyuric ($p < 0.05$) at 24 and 96 hr pi. Their basal urine osmolalities were 40 to 60% lower than control values ($p < 0.05$) from 24 to 72 hr pi. Furthermore, these animals were impaired in their ability to concentrate urine and conserve water. A 24-hr period of water deprivation, between 96 and 120 hr pi demonstrated that treated rats produced 70% more urine than controls ($p < 0.05$), and that urine osmolality of treated rats was a third lower ($p < 0.01$; Table 6). The Day-29 pups that were not weaned had lowered basal urine osmolalities 24 and 48 hr pi ($p < 0.001$), but slightly elevated osmolality 120 hr pi ($p < 0.05$). These animals were unable to increase urine osmolality to the extent of controls after a 4-hr period of water deprivation at any of the observation times ($p < 0.001$; Table 2).

Urinary pH, which had been slightly acidic in suckling pups, was close to neutral in control weanlings. The urine of $HgCl_2$-treated rats became more basic from 72 to 120 hr pi ($p < 0.01$). Urinary chloride content was significantly lower (30 to 65% of control values) from 24 to 120 hr pi ($p < 0.05$). Urinary protein levels were almost 10 times normal in treated rats 24 hr pi ($p < 0.001$) and more than twice the control value ($p < 0.05$) from 48 to 96 hr pi (Table 6).

Serum creatinine and urea nitrogen were increased significantly at 24 and 48 hr pi (Table 5). Albumin, but not total serum protein, was more than 10% lower in treated rats 24 and 48 hr pi. Serum GOT was increased but glucose was diminished ($p < 0.05$) at 24 hr pi. Total bilirubin, alkaline phosphatase, and GPT were unaffected by the treatment (Table 5).

All but one of the treated pups tested 24 hr pi were glucosuric. Approximately 64% of the treated pups were glucosuric 48 hr pi, and by 120 hr pi no glucose was detected in the urine of treated animals. At 24 hr pi, 78% of the treated rats had blood in their urine. This percentage decreased to 29% at 48 hr and to 0% at 120 hr pi. (Table 4).

All rats administered $HgCl_2$ on Day 29 had prominent renal lesions. Acute cortical tubular necrosis was widespread 24 and 48 hr pi. By 48 hr pi, epithelium in most proximal tubules

TABLE 6

EFFECTS OF INJECTION OF $HgCl_2$ ON RENAL PARAMETERS IN WEANLING RATS[a]

Hours post-injection	No. of animals	Body weight (g)	Water intake (ml/day)	Relative urine volume (ml/100 g body wt./day)	Urine osmolality (mOsM)	Urine pH	Urine chloride (meq/l)	Urine protein (mg/100 ml)
Control								
0	6	87.0 ± 4.4	12.7 ± 1.8	6.3 ± 1.1	1318 ± 200	7.42 ± 0.11	266 ± 48	13.11 ± 3.76
24		94.7 ± 5.5	19.2 ± 1.4	9.0 ± 1.1	1203 ± 134	7.02 ± 0.18	194 ± 19	8.89 ± 2.18
48		100.8 ± 6.3	19.2 ± 1.1	9.0 ± 1.4	1376 ± 258	7.10 ± 0.22	236 ± 35	17.60 ± 3.75
72		107.2 ± 7.0	19.2 ± 1.1	7.9 ± 0.8	1315 ± 128	7.08 ± 0.19	299 ± 26	20.73 ± 6.28
96		114.5 ± 7.7	17.7 ± 1.2	6.7 ± 0.9	1421 ± 141	7.05 ± 0.16	232 ± 24	19.8 ± 4.86
120		106 ± 6.9	[c]	4.2 ± 0.3	2052 ± 93	6.85 ± 0.11	364 ± 21	34.20 ± 3.10
$HgCl_2$								
0	6	79.7 ± 3.7	11.3 ± 2.4	5.6 ± 0.9	1482 ± 124	7.59 ± 0.17	306 ± 30	16.40 ± 2.33
24		78.7 ± 5.0[b]	15.6 ± 4.3	13.0 ± 3.3	679 ± 141[b]	6.99 ± 0.16	60 ± 12[d]	87.40 ± 25.60[d]
48		75.8 ± 7.0[d]	16.0 ± 3.7	9.8 ± 2.5	571 ± 117[c]	6.33 ± 0.16	81 ± 17[d]	44.96 ± 10.68[c]
72		79.2 ± 7.0[d]	17.8 ± 2.4	16.2 ± 3.4[c]	790 ± 150[b]	8.08 ± 0.33[c]	100 ± 23[c]	44.95 ± 9.93[b]
96		83.2 ± 7.8[d]	16.2 ± 2.0	12.7 ± 3.4[b]	1129 ± 312	8.63 ± 0.19[d]	151 ± 55[b]	41.85 ± 9.31[b]
120		75.8 ± 7.9[d]	[c]	7.2 ± 1.7	1379 ± 216[c]	8.31 ± 0.32[d]	226 ± 56[d]	29.75 ± 0.78

[a] Animals were weaned and placed in metabolism cages on Day 28. A 24-hr urine sample was collected from each rat (time 0 above) and they were injected at this time. All of the observations in a group are from the same six animals.

[b] Significantly different from controls by general linear models procedure, $p < 0.05$.

[c] Significantly different from controls by general linear models procedure, $p < 0.01$.

[d] Significantly different from controls by general linear models procedure, $p < 0.001$.

[e] 24-hr water deprivation.

in the majority of the rats were severely damaged (Fig. 3). Tubular regeneration had begun by 120 hr pi. Mitotic figures were frequently observed, and mineralized casts were present in the lumens of proximal tubules (Fig. 4).

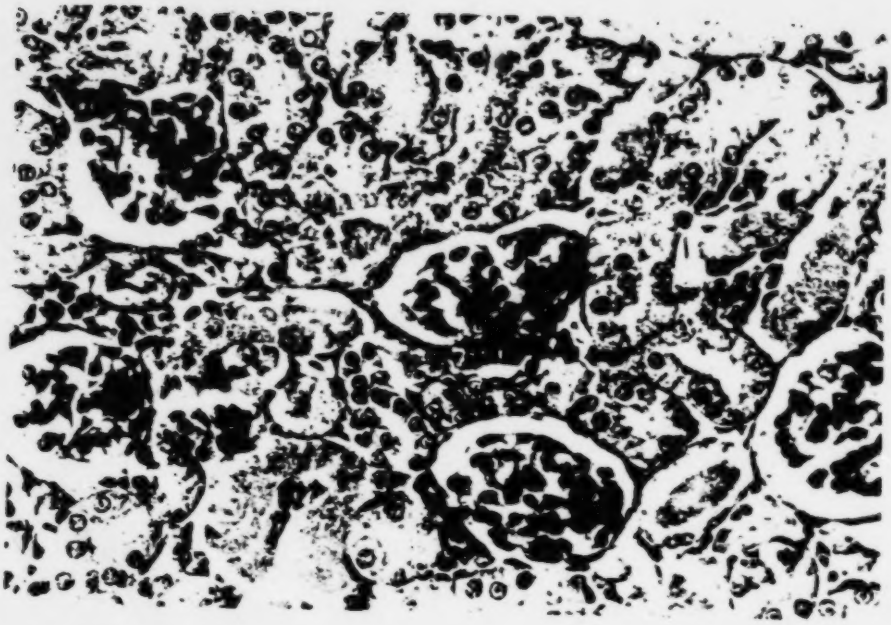
DISCUSSION

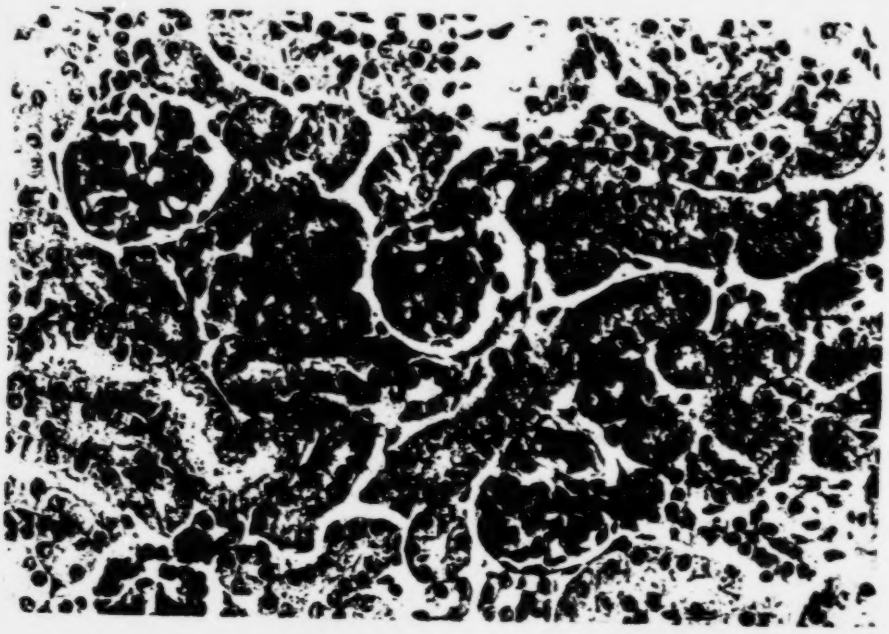
Mercuric chloride induced alterations in morphology and numerous renal functional parameters which were related in severity to the stage of maturation of the rats. Body weight was decreased, kidney weight was increased, and histopathologic changes in renal cortical tubules were observed in treated rats. Studies of compromised renal function following $HgCl_2$ exposure included polyuria, inability to excrete a concentrated urine, increased serum creatinine and urea nitrogen levels, glucosuria, proteinuria, and hematuria. In all cases, the severity of these toxic effects increased with increasing age of the rat. Neonates were largely insensitive to the administered dose of $HgCl_2$, whereas 29-day-old weanlings were the most affected. The observed increase in kidney weight was progressive, becoming most prominent 120 hr following treatment of the more mature rats. This effect was most likely due to interstitial edema, an effect often observed following exposure to $HgCl_2$ and other renal toxicants (Oken, 1981). The histopathologic changes induced by $HgCl_2$ in renal cortical tubules of adult rats have been observed previously by both light and electron microscopy (Ganote *et al.*, 1974; Preuss *et al.*, 1975; McDowell *et al.*, 1976; Haagsma and Pound, 1979, 1980; Trifillis *et al.*, 1981). The same patterns of histologic alterations observed in the present study were reported; namely, tubular dilatation, epithelial necrosis, and subsequent regeneration of the proximal tubular epithelium. In the present study, these effects were age related, with only the mildest lesions visible in neonates and more severe tubular necrosis in weanlings.

Renal functional alterations induced by $HgCl_2$ were also age related. In normal rats, the ability to produce a concentrated urine is initially low, then improves as the animals mature postnatally (Kavlock and Gray, 1982), a finding corroborated by the present study. Following $HgCl_2$ exposure, functional capacity decreased, as the ability to conserve water and to concentrate solutes in urine was compromised. Polyuria and a severely diminished urine osmolality after water deprivation were observed. These toxic manifestations were most severe at the oldest ages; however, it is important to note that polyuria was observed in treated neonates. Aside from a lowered urinary pH, this polyuria was the only functional effect produced by $HgCl_2$ exposure in the first week of life.

Normal changes in urinary protein content were observed. The high levels of urinary protein seen in young pups were probably due to an immature glomerular filtration barrier (Schaeverbeke and Cheignon, 1980). After glomerular maturation, protein spillage fell to minimal levels until Day 20, when protein again becomes a normal urinary constituent, especially in male rodents (Bardin and Catterall, 1981). Significant proteinuria was present at 120 hr pi in treated 8- and 15-day-olds, and at earlier observation times in animals injected on Day 22 and 29. The total quantity of urinary protein was even greater at the older ages, as a polyuria accompanied the increase in urine protein concentration. Interestingly, the proteinuria seen at 22 and 29 days was correlated with a drop in serum albumin levels. Other indicators of renal damage were also present, and in increasing frequency and severity with age. The frequency of individuals with hematuria and glucosuria increased. Serum creatinine and urea nitrogen levels increased. Serum transaminases were increased, probably reflecting cellular damage in the kidney.

The reason for the age-related increases in susceptibility to the nephrotoxic effects of $HgCl_2$ is unclear. Jugo (1977) has concluded that, given equivalent absorption in both young and adult animals, the toxicity of heavy metals is lower in the young than in the adult.





Thomas and Smith (1979) found that a sc dose of $HgCl_2$ was virtually totally retained in the suckling rat until Day 16, after which excretion of mercury began. They also noted that, despite the onset of mercury excretion, the fractional burden of mercury in the kidney increased. Similarly, Jugo (1975) reported that following iv $HgCl_2$ injection, the fraction of mercury retained by the kidney was lower in young than in adult rats. The functional capabilities of the immature rat kidney are considerably less developed than those of the adult. Glomerular filtration rate and filtration fraction are comparatively low (Horster and Lewy, 1970). This low rate may result in the reduced distribution of mercury noted by Jugo (1975). In the present study, we determined that several maturational events, notably a precipitous fall in serum creatinine levels (and probably a concomitant rise in creatinine clearance) occurred on or near Day 16. Thus, an increase in glomerular filtration rate may be a primary causative factor responsible for the increased excretion and renal uptake of inorganic mercury observed by Thomas and Smith (1979). The increase in renal mercury burden also appears to correlate with the increased nephrotoxicity observed in the present study.

An alternative explanation for the decreased sensitivity of neonates to nephrotoxic effects of $HgCl_2$ may involve the presence of the naturally occurring chelator, metallothionein. Metallothionein is a low molecular weight protein rich in cysteinyl residues which bind divalent metals, such as mercury (Kagi *et al.*, 1974). This protein has been found in higher concentrations in the perinatal rat (Bell, 1979; Oh and Whanger, 1979) than in the postweaning animal. Thus, the mature rat, with less available metallothionein, may be more susceptible to $HgCl_2$ intoxication than the neonate.

The pathogenesis of renal failure induced by mercury and other nephrotoxins has been extensively studied (for reviews, see Oken, 1976, 1981), although the mechanisms by which damage is produced remain unclear. It has been postulated that $HgCl_2$-induced renal failure is mediated by the renin-angiotensin system (Oken, 1976; Zalme *et al.*, 1976; Sato et al., 1977; Barnes *et al.*, 1980); however, recent studies have not corroborated this theory (Oken, 1981). Alexander and Grimason (1967) reported that the site of renin synthesis, the juxtaglomerular apparatus, is not histologically observable in the rat kidney until 2 weeks after birth, but Schmidt *et al.* (1972) have reported its presence in neonates. Furthermore, it has been demonstrated that plasma renin levels are higher in suckling than in postweaning rats (Pohlova and Jelinek, 1974). This evidence, combined with our findings, do not support the hypothesis that renal failure is mediated by the renin–angiotensin system.

In summary, the present study investigated the toxicity of $HgCl_2$ to the postnatally maturing rat kidney. For all parameters measured, neonates were less sensitive to $HgCl_2$. Sensitivity increased as the pups matured. These findings are in contrast to a previous study of the effects of $HgCl_2$ on the developing rat kidney (Bidani *et al.*, 1979). Using serum urea nitrogen as a measure of renal damage, they reported that recovery from $HgCl_2$ exposure was slower in 2-week-old rats as compared with 4- and 8-week-olds and that mortality was higher in the younger group. Corroborating the present study, Kavlock (1982)

FIG. 3. Photomicrographs of kidney cortex from control (bottom) and $HgCl_2$-treated (top) rats. The rats were killed 48 hr after injection on Day 29. The control kidney is histologically mature and normal. The section from $HgCl_2$-treated rat shows severe acute proximal tubular damage. The proximal tubular epithelium was largely necrotic, with a highly eosinophilic cytoplasm and extensive karyolysis. In many cases, the necrotic epithelium had sloughed from the basement membrane. Original magnification—200×; hematoxylin and eosin stain.

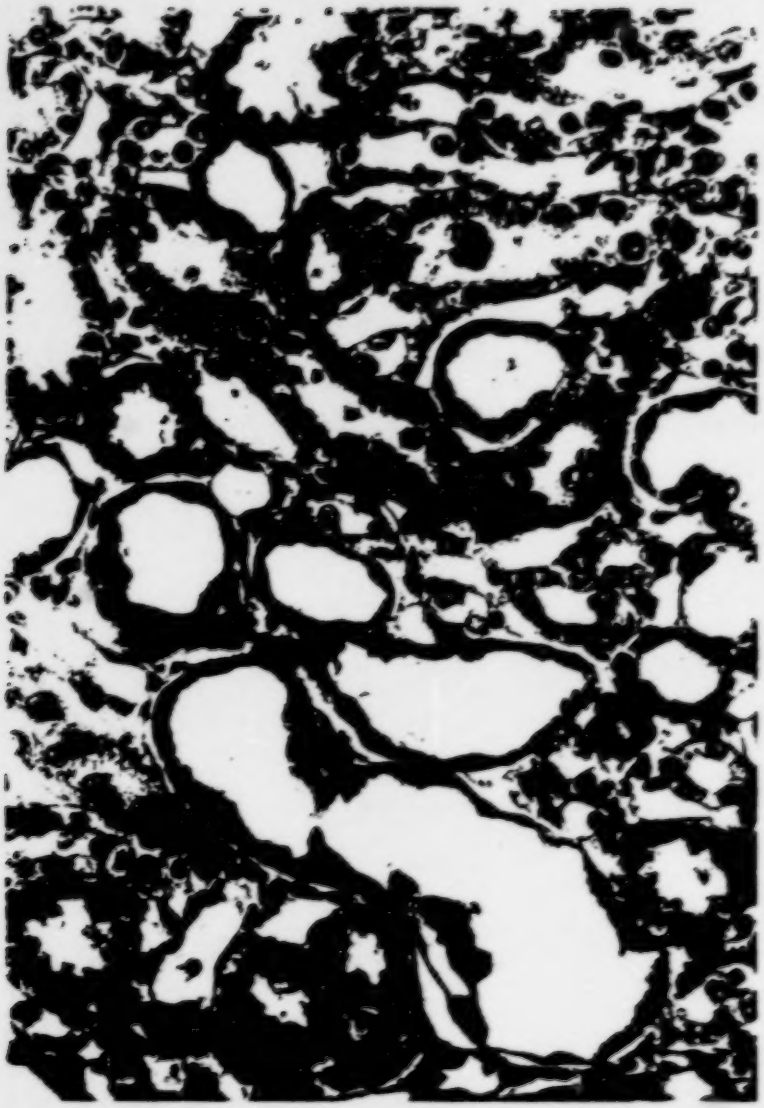


FIG. 4. Photomicrograph of kidney cortex from a $HgCl_2$-treated rat killed 120 hr after injection on Day 29. Tubular dilatation is prominent. Regeneration is extensive, characterized by squamous and low cuboidal cells lining the proximal tubular lumen. Debris from necrotic epithelial cells is present in some tubular lumens. Original magnification—200×; hematoxylin and eosin stain.

detected no $HgCl_2$-induced renal toxicity in 3-, 6- or 10-day-old rats when treated on postnatal Days 1 and 2. The neonatal rat kidney appears to be relatively insensitive to $HgCl_2$-induced nephrotoxicity. Toxicity was enhanced as the animals matured.

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